Supporting Information

Discovery of Potent and Selective Covalent Protein Arginine Methyltransferase 5 (PRMT5) Inhibitors.

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Flash plate assay. Compounds were solubilized, and 3-fold diluted in 100% DMSO. These diluted compounds were further diluted in the assay buffer (20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.002% Tween20, 1 mM TCEP, 1% DMSO) for 10-dose IC50 mode at a concentration 10-fold greater than the desired assay concentration. Standard reactions were performed in a total volume of 30 µl in assay buffer, with 300 nM histone H4 based AcH4-23 (Anaspec: AS-65002) as substrate. To this was added the PRMT5/MEP50 complex diluted to provide a final assay concentration of 2.5 nM and the compounds were allowed to preincubate for 20 minutes at 37 °C. The reaction was initiated by adding S-[3H-methyl]-adenosyl-L-methionine (PerkinElmer: NET155001MC) to final concentration of 1 µM. Following a 30 minutes incubation at 37 °C, the reaction was stopped by adding 25 µL of 8M Guanidine HCl. Prepare streptavidin YSI SPA beads (Perkinelmer: RPNQ0012) at 0.3 mg/mL in assay buffer. To each reaction, add 150 µL of SPA beads suspension, and incubated while shaking at room temperature for 30 minutes. The plate was centrifuged at 100 xg for 30 second before reading in a scintillation counter. IC50 values were determined by fitting the data to the standard 4 parameters with Hill Slope using GraphPad Prism software.

Jump-dilution assay. The PRMT5/MEP50 complex (0.1 µM) was incubated with inhibitor (2 µM compound 9) for 30 min at 37 °C and dialyzed in 500 mL dialysis buffer. The resulting protein-inhibitor complex was rapidly diluted 50-fold in the assay buffer containing 300 nM biotinylated AcH4-23 peptide and 1 µM 3H-SAM. The free enzyme control was diluted in the absence of the inhibitor. The background absorbance control omitted the enzyme. After dilution, PRMT5 activity was monitored by flash plate assay at 37 °C for 1 hour, and a 20 µL aliquot of the reaction mixture was quenched by 25 µL of 8M guanidine HCl at varied time points (0, 3, 6, 9, 12, 15, 20, 30, 40, 50, 60 min). Data were fit to following equation to obtain k_{off}.

$$P = V_s t + (V_0 - V_s)\frac{(1 - e^{-kt})}{k}$$

In the off-rate experiment, V_0 represents fully inhibited enzyme velocity, and it was estimated using a reaction with no enzyme. V_S represents the uninhibited enzyme velocity, and it was measured in a reaction with the PRMT5/MEP50 complex and no inhibitor. The k_{off} value is reported as ± SD from three separate determinations.

Time-dependent inactivation assay. Time dependent inactivation of the PRMT5/MEP50 complex was also performed in format. Briefly, 1 nM of PRMT5/MEP50 complex was incubated with AcH4-23 (300 nM), 3H-SAM (1 µM) and PRT1000277 in varied concentration (0, 5, 10, 25, 50, 100, 250, and 500 nM final) at 37 °C. The reactions were initiated by addition of the PRMT5/MEP50 complex. Aliquots (20 uL) were removed at various time point (0, 5, 10, 20, 30, 45, 60, 75, 90, 105, 120 min), and quenched with 8M guanidine HCl. The methyltransferase activity was monitored by flash plate assay as described above. The initial reaction rates at various inhibition concentrations were determined by fitting data to following equation:

$$P = \frac{V_0(1 - e^{-kt})}{k}$$

V0 is the initial velocity of product formation. The resulting k_{obs} was further plots against the concentration of inactivator to yield hyperbolic profiles for saturable two-step binding. Nonlinear regression analysis was utilized to estimate the K_I, and k_{inact}.

$$k_{obs} = \frac{k_{inact}[I]}{K_I + [I]}$$

pKa determination of Cysteine449 in SAM binding pocket. The pH dependence of inactivation of PRMT5 by IAM was determined out as described previously (ref. 1). Briefly, PRMT5 alone (0.5 μM) was preincubated in the following buffers with or without IAM (0.5 mM) for 3 min: sodium citrate at pH 3.0 4.0; sodium acetate at pH 5.0; sodium phosphate buffer at pH 6.0; HEPES buffer at pH 7.0, Tris buffer at pH 8.0, 9.0 and sodium bicarbonate buffer at pH 9.4. All buffers were at a concentration of 10 mM, and the ionic strength was adjusted to 0.5 M in all cases by addition of the appropriate amount of NaCl. Following preincubation, PRMT5 activity was determined by adding an aliquot (6 μL) of the preincubation mixture to the substrate mixture containing GAR (0.05 mg/mL) and 3-H SAM in Flash-plate assay. Percent activity remaining was calculated by dividing rates of methylation after preincubation with IAM by rates in the absence of IAM and multiplying the result by 100%. Percent activity was plotted versus pH, and an adaptation of the Henderson-Hasselbalch equation was used to solve for pKa:

$$\%Activity = 100 - 100 \times \left(\frac{10^{pH-pKa}}{1 + 10^{pH-pKa}}\right)$$

Cell treatment and Western Blotting for detecting Symmetric Di-Methyl Arginine (sDMA) marks

Compound titration and cell culture: Compounds were dissolved in DMSO to make 10 mM stock and 3-fold series dilutions were further conducted to make working stocks top at 1 mM. Granta-519 cells were maintained in PRMI 1640 (Corning Cellgro, Catalog #: 10-040-CV) supplemented with 10% v/v FBS (GE Healthcare, Catalog #: SH30910.03).

To determine enzyme inhibition IC_{50} values in Granta-519 cells using Western Blot analysis. One day before experiment, Granta-519 cells were passaged to a density of $0.5x10^6$ cells/ml. The next day, Granta-519 cells were spun down at 1,500 rpm for 4 min, resuspend in fresh medium at $0.5x10^6$ cells/ml and 3 mL of culture ($1.5x10^6$ cells) were seeded into 6 well plate. Eight-point, 3-fold serial dilutions of compound working stocks were added to cells (3 μl, 1:1,000 dilution, DMSO concentration was 0.1%; final top concentration at 1 uM) and incubated for 3 days. Cells incubated with DMSO was used as a vehicle control.

Cells were harvested 3 days later, resuspended in 15 uL PBS, lysed in 4% SDS, and homogenized by passing through homogenizer column (Omega Biotek, Catalog #:

HCR003). Total protein concentrations were determined by BCA assay (ThermoFisher Scientific, Catalog #: 23225). Lysates were mixed with 5x Laemmli buffer and boiled for 5 min. Forty ug of total protein was separated on SDS-PAGE gels (Bio-Rad, catalog #: 4568083, 4568043), transferred to PVDF membrane, blocked with 5% dry milk (Bio-Rad, Catalog #: 1706404) in TBS with 0.1% v/v Tween 20 (TBST) for 1 hour at room temperature (RT), and incubated with primary antibodies (sDMA: Cell signaling, Catalog #: 13222, 1:3,000; β-Actin: sigma, Catalog #: A2228, 1:5,000) in 5% dry milk in TBST at 4 °C overnight. The next day, membranes were washed with TBST, 5 x 5 min, and incubated with HRP conjugated seconded antibody (GE Healthcare; Catalog #: NA934-1ML, NA931-1ML; 1:5,000) for 2 hours at RT, followed by 5 x 5 min washes with TBST, and incubation with ECL substrates (Bio-Rad, Catalog #: 1705061, 1705062). Chemiluminescent signal was captured with Fluochem HD2 imager (Proteinsimple). SmD3me2s bands were quantified by ImageJ. Signals were normalized to β-Actin and DMSO control. IC_{50} values were calculated using Graphpad Prism ([Inhibitor] vs. normalized response – Variable slope).

Cell proliferation assay to determine IC_{50} in Granta-519

One day before experiment, Granta-519 cells were passaged to a density of $0.5x10^6$ cells/ml. On the day of experiment (day 0), Granta-519 Cells were spun down at 1,500 rpm for 4 min, resuspended in fresh medium to $0.5x10^6$ cells/ml and 190 µl of cells were added to 96 well plates. Compound working stocks were first diluted at 1:50 with fresh medium in 96 well plate and10 µL of diluted drugs were added to 96 well plates containing cells and incubated for 3 days. DMSO was used a vehicle control.

One day 3, cells were resuspended and 50 µL of Granta-519 cells were transferred to a new 96-well plate containing 140 µL fresh medium. Compound working stocks were freshly diluted at 1:50 with medium and 10 µL of diluted drugs were added to cells and incubate for 3 more days. The same process was repeated on day 6. Cells were allowed to grow for additional 4 days.

On day 10, cells were resuspended and 100 µL Granta-519 cells were transferred to a new 96 well plate containing 10 µL of Cell Counting Kit-8 (CCK-8, Jojindo, CK04-13) solution. Plates were incubated in CO_2 incubator for 2 hours (Granta-519 cells) and OD_{450} values were measured with a microplate reader (iMark microplate reader, Bio-Rad). Percentage of viable cells, relative to DMSO vehicle control, were calculated and plotted in Graphpad Prism ([Inhibitor] vs. normalized response – Variable slope) to determine proliferation IC_{50} values on day 10

PRMT5/MEP50 protein expression, purification and crystallization

The human PRMT5 and MEP50 were cloned, co-expressed and purified as described (ref. 2). Briefly, the two constructs were prepared and standard baculovirus expression using Bac-to-Bac protocol (Life Technologies) was used to generate viruses for them. Co-expression of PRMT5:MEP50 (at 1:2 ratio) in High Five cells were conducted. Cells

were harvested after 48 hour infection by centrifugation and pellets were used for purification.

To yield the protein complex for crystallization, three steps purification was conducted, Ni-NTA(QIAGEN) followed by anti-FLAG resin and superdex 200(HiLoad 16/60, GE lifesciences). The final protein was in a buffer composing of 10 mM HEPES, 150mM NaCl, 1 mM TCEP, 10%glycerol, pH 8.0 and concentrated to 16mg/ml for crystallization.

Prior crystallization, PRMT5:MEP50 complex at 15mg/ml was mixed with 2 mM compound **10** (from 100mM DMSO stock) and incubated on ice for 2 hrs. After commercial kits screening and optimization, high quality crystals were obtained by hanging drop vapor diffusion drops with 2 uL of PRMT5:MEP50: compound **10** mixed with 2 uL of crystallization well solution of 0.1M Na-Citrate, 0.2M NaAc, 10% PEG4000, pH6.0.The crystals were cryo-protected in the mother liquid with 25 % ethylene glycol and flash frozen in liquid nitrogen.

PRMT5/MEP50:compound 10 co-crystal structure data collection, processing and structure refinement (PDB: 6K1S)

The dataset of PRMT5:MEP50: compound **10** crystal was collected at the Shanghai Synchrotron Radiation Facility (SSRF) beamline BL19u1 equipped with a DECTRIS PILATUS detector and processed with HKL3000 (ref. 3). Molecular replacement was performed using the co-ordinates from PDB entry 4GQB as a search model using Phaser (ref. 4) in CCP4 Suite (ref. 5). The compound restraints and coordinates were generated by ProDRG. Iterative manual model building and refinement were conducted using COOT (ref. 6) and REFMAC5 (ref. 7), respectively. The data collection and structure refinement statistics are summarized in Table S1.

Table S1. Data collection and refinement statistics.

	PRMT5/MEP50/compound **10**
Wavelength	0.97853
Resolution range	44.76 - 2.60 (2.69 - 2.60)
Space group	I 2 2 2
Unit cell	102.639 138.724 178.022 90 90 90
Total reflections	238029(14384)
Unique reflections	37407 (2714)

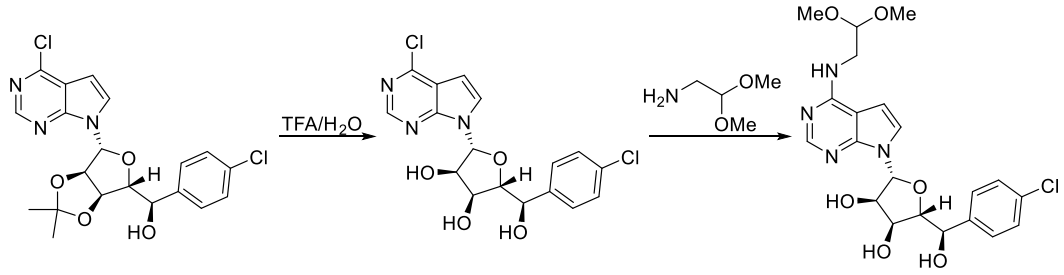
Multiplicity	6.4(5.3)
Completeness (%)	93.4(69.1)
Mean I/sigma(I)	19(2)
Wilson B-factor	64.51
R-merge	0.093(0.631)
Reflections used in refinement	37379 (2555)
Reflections used for R-free	1876 (145)
R-work	0.2072 (0.4034)
R-free	0.2785 (0.4986)
Number of non-hydrogen atoms	7554
macromolecules	7387
ligands	66
solvent	101
Protein residues	932
RMS(bonds)	0.014
RMS(angles)	1.83
Ramachandran favored (%)	92.20
Ramachandran allowed (%)	6.72

Ramachandran outliers (%)	1.08
Rotamer outliers (%)	2.56
Clashscore	4.83
Average B-factor	76.20
macromolecules	76.50
ligands	70.81
solvent	57.45

Statistics for the highest-resolution shell are shown in parentheses.

Synthetic procedures

Compound 6. (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(4-((2,2-dimethoxyethyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (6)



a) (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (**5**)

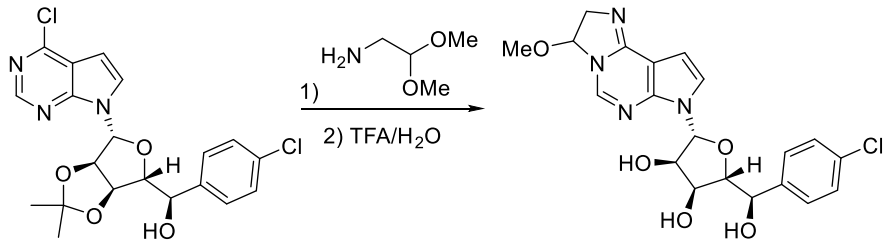
A 50 mL RBF and septum containing (R)-[(3aR,4R,6R,6aR)-4-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-6-yl]-(4-chlorophenyl)methanol (**4**, Ref. PCT Int. Appl., 2016178870) (455 mg, 1.04 mmol) was charged with a RT mixture of 2,2,2-trifluoroacetic acid (2.5 mL, 32.45 mmol) and Water (2.5 mL), sonicated for 10 s, purged with Ar, and stirred at RT for 2 h. The reaction mixture was concentrated under reduced pressure to remove the water and most of the TFA. The reaction was then diluted in MeOH (20 mL), and quenched with Amberlite IRA-67 until a neutral pH was obtained. The mixture was then filtered through a cotton plug, rinsed with additional MeOH and DCM, and concentrated under reduced pressure

to light brown foam. The crude product was purified by FCC (40g SiO_2, 3 to 4% MeOH in DCM, wet-loaded in DCM) to yield (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (**5**) (128 mg, 0.31 mmol, 30.0% yield) as a white powder. Rf = 0.26 (3% MeOH in DCM). LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{17}H_{16}Cl_2N_3O_4$: 396.051. Found: 396.0. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.02 (d, *J* = 3.8 Hz, 1H), 7.44 – 7.32 (m, 4H), 6.79 (d, *J* = 3.7 Hz, 1H), 6.19 (d, *J* = 7.7 Hz, 1H), 6.02 (d, *J* = 4.1 Hz, 1H), 5.39 (s, 1H), 5.21 (d, *J* = 4.0 Hz, 1H), 4.80 (t, *J* = 4.1 Hz, 1H), 4.58 (s, 1H), 4.12 (t, *J* = 3.3 Hz, 1H), 4.00 (dd, *J* = 5.3, 1.3 Hz, 1H). ^{1}H NMR of (400 MHz, DMSO-d_6+D_2O) δ 8.66 – 8.60 (m, 1H), 7.96 – 7.87 (m, 1H), 7.42 – 7.29 (m, 4H), 6.76 (t, *J* = 3.1 Hz, 1H), 6.15 (dd, *J* = 7.6, 3.5 Hz, 1H), 4.76 (t, *J* = 3.9 Hz, 1H), 4.55 (t, *J* = 6.5 Hz, 1H), 4.11 (d, *J* = 5.1 Hz, 1H), 4.01 (dd, *J* = 5.1, 1.1 Hz, 1H).

b) (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(4-((2,2-dimethoxyethyl)amino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (**6**)

A 4 mL vial containing a mixture of (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (**5**) (21 mg, 0.050 mmol) and 2,2-dimethoxyethanamine (0.03 mL, 0.28 mmol) in 1,4-Dioxane (0.4 mL) and IPA (0.1 mL) was purged with Ar, sealed, and heated at 100 °C for 10 h. TLC showed some SM remained. The mixture was concentrated under reduced pressure and purified by FCC (4g SiO_2, 3->5% MeOH in DCM, wet-loaded in DCM). Fractions containing product were concentrated under reduced pressure and heat (50 °C) to yield (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-[4-(2,2-dimethoxyethylamino)pyrrolo[2,3-d]pyrimidin-7-yl]tetrahydrofuran-3,4-diol (**6**) (18.1 mg, 0.038 mmol, 72% yield) as a white powder. LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{21}H_{26}ClN_4O_6$: 465.154. Found: 465.0. Rf = 0.5 (10% MeOH in DCM). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.75 (t, *J* = 6.0 Hz, 1H), 7.47 – 7.36 (m, 4H), 7.35 (d, *J* = 3.7 Hz, 1H), 6.68 (d, *J* = 3.6 Hz, 1H), 6.64 (d, *J* = 3.3 Hz, 1H), 5.92 (d, *J* = 7.9 Hz, 1H), 5.23 (d, *J* = 6.3 Hz, 1H), 5.04 (d, *J* = 3.7 Hz, 1H), 4.81 (t, *J* = 3.6 Hz, 1H), 4.62 (q, *J* = 6.1 Hz, 1H), 4.58 (t, *J* = 5.4 Hz, 1H), 4.04 – 3.97 (m, 2H), 3.58 (t, *J* = 5.7 Hz, 2H), 3.30 (s, 6H).

Compound 8. (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(3-methoxy-2,3-dihydro-7H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (8)



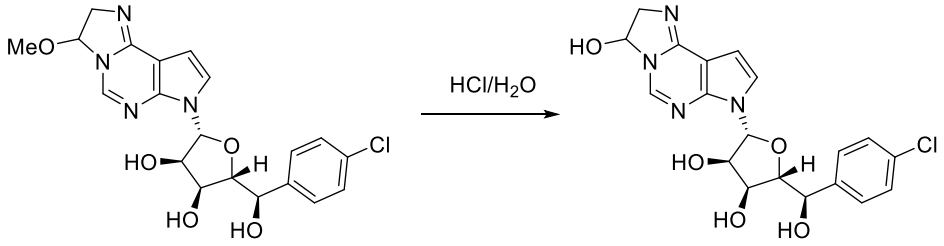
Compound 4 **Compound 8**

2,2-Dimethoxyethanamine (0.1mL, 0.92 mmol) was added to a mixture of (R)-[(3aR,4R,6R,6aR)-4-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-6-yl]-(4-chlorophenyl)methanol (**4**, Ref. PCT Int. Appl., 2016178870) (80 mg, 0.18 mmol) in IPA (1 mL), sealed and heated at 100 °C for 2 h. TLC showed a more polar product and small amount of unreacted starting material.

The reaction mixture was stirred at RT over 72 h, concentrated, and the residue was purified on a 4g column, which was eluted with 0-50% EA/hexane to give ~80 mg of foamy white intermediate (**7**).

Hydrolysis of the intermediate (**7**) was carried out in trifluoracetic acid (0.5 mL, 0.18 mmol) and Water (0.05 mL) at 0 °C. After 2 h, TLC showed the completion of the reaction (9:1 DCM/MeOH) and formation of a clean polar spot. The reaction mixture was concentrated, taken into MeOH, cooled to 0 °C, and neutralized with concentrated NH_4OH to pH 9. The resulting mixture was concentrated and purified on a 4g column, which was eluted with 0-14% MeOH/DCM to give a TFA salt of (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-(3-methoxy-2,3-dihydroimidazo[1,2-c]pyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (**8**) (66 mg, 66% yield), as a white solid. LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{20}H_{22}ClN_4O_5$: 433.12/435.12; Found: 433.0/435.0; ^{19}FNMR -75 ppm; ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) (mixture of diastereomers) δ 8.89/8.88 (s, 1H), 7.84/7.83 (s, 1H), 7.42-7.36 (m, 4H), 6.88-6.86 (m, 1H), 6.30-6.27 (m, 1H), 6.16-6.13 (m, 1H), 4.77 (d, *J* = 5.3 Hz, 1H), 4.51-4.46 (m, 1H), 4.17-4.11 (m, *J* = 12.2, 2H), 4.05 – 3.96 (m, 2H), 3.46/3.45 (s, 3H).

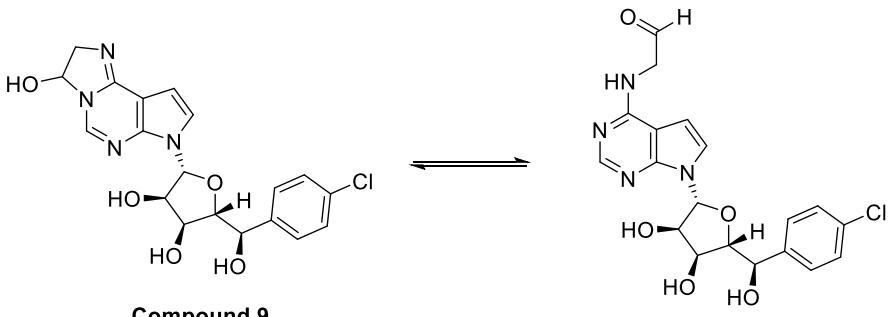
Compound 9. (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(3-hydroxy-2,3-dihydro-7H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (9)



A mixture of (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-(3-methoxy-2,3-dihydroimidazo[1,2-c]pyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (**8**) (45 mg, 0.082 mmol) and 1N HCl (0.5 mL, 0.50 mmol) was heated at 100 °C for 2 h. LCMS showed completion of hydrolysis of compound **8**. The reaction mixture was concentrated under vacuum to give an HCl salt of (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(3-hydroxy-2,3-dihydro-7H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (**9**) (39 mg, 0.08 mmol, 100% yield) as a white solid. LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{19}H_{20}ClN_4O_5$·HCl: 419.10/421.10; Found: 419.1/421.5. ^{1}H NMR (400 MHz, DMSO- d_6 + D_2O) (mixture of diastereomers) δ 8.73/8.72 (s, 1H), 7.86-7.84 (m, 1H), 7.43-7.36 (m, 4H), 6.90-6.89 (m, 1H), 6.48 – 6.41

(m, 1H), 6.16-6.13 (m, 1H), 4.78 (d, *J* = 5.3 Hz, 1H), 4.51-4.46 (m, 1H), 4.23-4.18 (m, 1H), 4.11 (d, *J* = 4.9 Hz, 1H), 4.01 (dd, *J* = 5.3, 1.7 Hz, 1H), 3.82-3.78 (m, 1H)

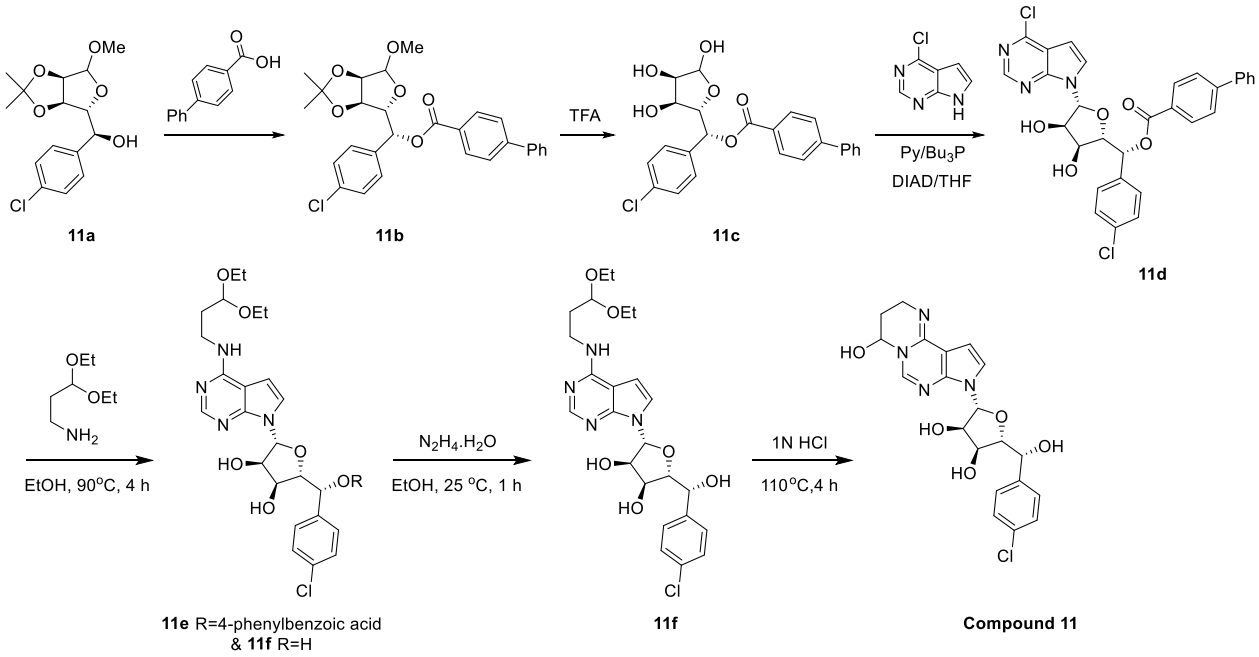
Compound 10. 2-((7-((2R,3R,4S,5R)-5-((R)-(4-chlorophenyl)(hydroxy)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)acetaldehyde (10)



Compound 9 Compound 10

An HCl salt of (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(3-hydroxy-2,3-dihydro-7H-imidazo[1,2-c]pyrrolo[3,2-e]pyrimidin-7-yl)tetrahydrofuran-3,4-diol (**9**) (19 mg, 0.043 mmol,) was dissolved in a mixture of 0.3 mL of MeOH and 1 mL of H_2O, pH ~4-5, Amberlite IRA-67 free base was added until pH ~8. The resulting mixture was stirred at RT for 30 min, filtered through a cotton ball in a pipette, concentrated to give 2-((7-((2R,3R,4S,5R)-5-((R)-(4-chlorophenyl)(hydroxy)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)acetaldehyde (**10**) (11 mg, 0.026 mmol, 62% yield) as a white solid. LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{19}H_{20}ClN_4O_5$: 419.10/421.10; Found: 419.0/421.0; ^{1}H NMR (400 MHz, DMSO- d_6 + D_2O) δ 9.59 (s, 1H), 8.09 (s, 1H), 7.42-7.35 (m, 5H), 6.64 (s, 1H), 5.93-5.87 (m, 1H), 4.79 (d, *J* = 4.2 Hz, 1H), 4.58 (t, *J* = 6.3 Hz, 1H), 4.25 (m, 1H), 4.02-4.00 (m, 3H).

Compound 11. (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(4-hydroxy-3,4-dihydropyrimido[1,2-c]pyrrolo[3,2-e]pyrimidin-8(2H)-yl)tetrahydrofuran-3,4-diol (11)



a) Synthesis of [(R)-[(3aR,4R,6R,6aR)-4-methoxy-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-6-yl]-(4-chlorophenyl)methyl] 4-phenylbenzoate (**11b**)

To a solution of (S)-[(3aR,4R,6R,6aR)-4-methoxy-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-6-yl]-(4-chlorophenyl)methanol (**11a**) (28.4 g, 90.2 mmol) in Toluene (300 mL) was added 4-phenylbenzoic acid (27.0 g, 136 mmol), and triphenylphosphine (35.67 g, 136.0 mmol) at 0 °C. The reaction mixture was stirred at 0 °C. (E)-diisopropyl diazene-1,2-dicarboxylate (27.5 g, 136 mmol) was added dropwise at 0 °C, stirred for 30 min at the same temperature and continued for 2 h at 25 °C. The reaction mixture was filtered, and the filtrates were concentrated under reduced pressure. The crude product was purified by silica chromatography (PE: EA = 50:1) to give [(R)-[(3aR,4R,6R,6aR)-4-methoxy-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-6-yl]-(4-chlorophenyl)methyl] 4-phenylbenzoate (**11b**) (40.0 g, 80.8 mmol, 89.6% yield) as a white solid.

b) [(R)-(4-chlorophenyl)-[(2S,3S,4R)-3,4,5-trihydroxytetrahydrofuran-2-yl]methyl] 4-phenylbenzoate (**11c**)

A solution of [(R)-[(3aR,6R11c,6aR)-4-methoxy-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-d][1,3]dioxol-6-yl]-(4-chlorophenyl)methyl] 4-phenylbenzoate (**11b**) (36.4 g, 73.5 mmol) in 2,2,2-trifluoroacetic acid (2 L) and Water (1 L) was stirred at 25 °C for 30 h. LCMS showed the reaction was complete. The solution was neutralized with NaOH aqueous solution to pH 7-8, extracted with EA 3 times, the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give crude product, which was purified by silica gel column chromatography (PE:EA=10:1 to PE:EA=1:1) to give [(R)-(4-

chlorophenyl)-[(2S,3S,4R)-3,4,5-trihydroxytetrahydrofuran-2-yl]methyl] 4-phenylbenzoate (**11c**) (29 g, 93% purity, 61 mmol, 83% yield) as a white solid. ^{1}H NMR (400 M Hz, DMSO-*d6*): δ 8.13 (d, *J* = 8.4 Hz, 2 H), 7.85 (d, *J* = 8.0 Hz, 2 H), 7.75 (d, *J* = 7.6 Hz, 2 H), 7.54-7.41 (m, 7 H), 6.40 (d, *J* = 4.4 Hz, 1 H), 6.01-5.97 (m, 1 H), 4.98-4.81 (m, 3 H), 4.17-4.10 (m, 2 H), 3.67 (d, *J* = 3.6 Hz, 1 H).

c) Synthesis of [(R)-(4-chlorophenyl)-[(2S,3S,4R,5R)-5-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl]methyl] 4-phenylbenzoate (**11d**)

To a solution of 4-Chloro-7H-pyrrolo[2,3-d]pyrimidine (810 mg, 5.27 mmol) in dry THF (40 mL) was added pyridine (0.43 mL, 5.27 mmol), Then tributylphosphine (2.63 mL, 10.6 mmol) and DIAD (2.18 mL, 11.1 mmol) was added at 30 °C, [(R)-(4-chlorophenyl)-[(2S,3S,4R)-3,4,5-trihydroxytetrahydrofuran-2-yl]methyl] 4-phenylbenzoate (**11c**) (2.50 g, 93% purity, 5.27 mmol) was added at once. The reaction mixture was stirred at 30 °C for 2h. LCMS showed the reaction was completed. The reaction mixture was concentrated and the crude product was purified by Prep-HPLC eluting with H2O:CH3CN from 85:15 to 5:95 to give [(R)-(4-chlorophenyl)-[(2S,3S,4R,5R)-5-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl]methyl] 4-phenylbenzoate (**11d**) (875 mg, 1.52 mmol, 28.8% yield) as a pale yellow solid. LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{30}H_{24}Cl_2N_3O_5$: 576.10/578.10; Found: 576.2/578.2

d) Synthesis of Compound **11e**

To a solution of [(R)-(4-chlorophenyl)-[(2S,3S,4R,5R)-5-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl]methyl] 4-phenylbenzoate (**11d**) (400 mg, 0.69 mmol) in Ethanol (2 mL), 3,3-diethoxypropan-1-amine (2.0 mL, 12.4 mmol) was added. The mixture was stirred at 80 °C for 4 h. LCMS indicated the reaction was completed and formation of the mixture of **11e** and **11f**. The mixture of crude products was used directly for the next step.

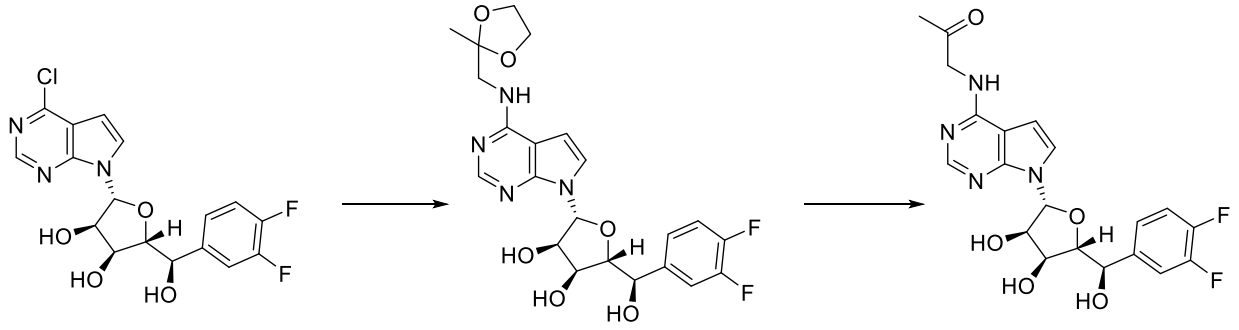
e) Synthesis of (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-[4-(3,3-diethoxypropylamino)pyrrolo[2,3-d]pyrimidin-7-yl]tetrahydrofuran-3,4-diol (**11f**)

To the reaction mixture of compound **11e** and **11f** (both [(R)-(4-chlorophenyl)-[(2S,3S,4R,5R)-5-[4-(3,3-diethoxypropylamino)pyrrolo[2,3-d]pyrimidin-7-yl]-3,4-dihydroxy-tetrahydrofuran-2-yl]methyl] 4-phenylbenzoate and (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-[4-(3,3-diethoxypropylamino)pyrrolo[2,3-d]pyrimidin-7-yl]tetrahydrofuran-3,4-diol were contained), hydrazine hydrate (2.0 mL, 41.2 mmol) was added. The mixture was stirred at 25 °C for 4 h. The mixture was purified by reverse phase Chem-flash eluting with CH_3CN/H_2O from 10/90 to 90/10 to give (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-[4-(3,3-diethoxypropylamino)pyrrolo[2,3-d]pyrimidin-7-yl]tetrahydrofuran-3,4-diol (**11f**) (297 mg, 0.557 mmol, 85.2% yield) (total yield over two steps) as a yellow solid. LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{24}H_{32}ClN_4O_6$: 507.19; Found: 507.1

f) Synthesis of (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(4-hydroxy-3,4-dihydropyrimido[1,2-c]pyrrolo[3,2-e]pyrimidin-8(2H)-yl)tetrahydrofuran-3,4-diol hydrochloride (**11**)

To a solution of (2R,3S,4R,5R)-2-[(R)-(4-chlorophenyl)-hydroxy-methyl]-5-[4-(3,3-diethoxypropylamino)pyrrolo[2,3-d]pyrimidin-7-yl]tetrahydrofuran-3,4-diol (**11f**) (250 mg, 0.47 mmol) in THF (1 mL), 1 M HCl (2 ml) was added. The mixture was stirred at 105 °C for 1 h. LCMS indicated the reaction has been completed. The mixture was purified by Prep-HPLC eluting with by Prep-HPLC eluting with CH_3CN/H_2O (0.1% TFA contained) from 5/95 to 95/5. The fractions were combined, concentrated and the residue was treated with 1N HCl, concentrated to give (2R,3S,4R,5R)-2-((R)-(4-chlorophenyl)(hydroxy)methyl)-5-(4-hydroxy-3,4-dihydropyrimido[1,2-c]pyrrolo[3,2-e]pyrimidin-8(2H)-yl)tetrahydrofuran-3,4-diol hydrochloride (**11**) (100 mg, 0.212 mmol, 45.2% yield) as a white solid. ^{1}H NMR (1:1 mixture of two isomers) (400 M Hz, DMSO-d6): δ 10.45 (s, 1 H), 8.48 (s, 1 H), 7.89 (s, 1 H), 7.76 (t, *J* = 3.2 Hz, 1 H), 7.36-7.42 (m, 4 H), 6.99 (d, *J* = 3.2 Hz, 1 H), 6.05-6.09 (m, 2 H), 5.97 (s, 1 H), 5.38 (s, 1 H), 5.24 (s, 1 H), 4.77 (d, *J* = 4.0 Hz, 1 H), 4.47-4.48 (m, 1 H), 4.12 (s, 1 H), 4.00 (s, 1 H), 3.57-3.63 (m, 2 H), 2.22-2.26 (m, 1 H), 2.14-2.16 (m, 1 H). ^{1}H NMR (400 M Hz, DMSO-d6 + D_2O): δ 8.42 (s, 1 H), 7.69-7.71 (m, 1 H), 7.36-7.42 (m, 4 H), 6.95 (d, *J* = 2.8 Hz, 1 H), 6.09 (dd, J_1 = 7.6 Hz, 1H, two peaks from 2 isomers), 6.03 (s, 1 H), 4.76 (d, *J* = 5.2 Hz, 1 H), 4.47-4.52 (m, 1 H), 4.15 (d, *J* = 4.8 Hz, 1 H), 4.04 (d, *J* = 5.6 Hz, 1 H), 3.60-3.64 (m, 2 H), 2.25-2.29 (m, 1 H), 2.10-2.19 (m, 1 H). LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{20}H_{22}ClN_4O_5$: 433.12/435.12; Found: 433.1/435.1.

Compound 12. 1-((7-((2R,3R,4S,5R)-5-((R)-(3,4-difluorophenyl)(hydroxy)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)propan-2-one (12)



12a **12b** **Compound 12**

a) Preparation of (2R,3S,4R,5R)-2-[(R)-(3,4-difluorophenyl)-hydroxy-methyl]-5-[4-[(2-methyl-1,3-dioxolan-2-yl)methylamino]pyrrolo[2,3-d]pyrimidin-7-yl]tetrahydrofuran-3,4-diol (**12b**)

A 2 mL microwave vial containing (2R,3R,4S,5R)-2-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)-5-[(R)-(3,4-difluorophenyl)-hydroxy-methyl]tetrahydrofuran-3,4-diol (50.mg, 0.13 mmol), 1-(2-Methyl-1,3-dioxolan-2-yl)methanamine (**12a**, prepared similar to that of

compound **5**) (55 mg, 0.47 mmol), and N-ethyl-N-isopropyl-propan-2-amine (0.05 mL, 0.29 mmol) was charged with 1-Propanol (1 mL) and sparged with Ar for 2 min The vial was then heated in a microwave reactor at 110 °C for 2 h 20 min. The reaction mixture (clear solution) was concentrated under reduced pressure and purified by FCC (12 g SiO2, 0->6% MeOH in DCM, wet-loaded in eluent) to yield (2R,3S,4R,5R)-2-[(R)-(3,4-difluorophenyl)-hydroxy-methyl]-5-[4-[(2-methyl-1,3-dioxolan-2-yl)methylamino]pyrrolo[2,3-d]pyrimidin-7-yl]tetrahydrofuran-3,4-diol (**12b**) (57 mg, 0.12 mmol, 95% yield) as a thick oil. Rf = 0.58 (2:1 Hexane:EtOAc); LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{22}H_{24}F_2N_4O_6$: 479.17; Found: 479.2.

b) Preparation of 1-[[7-[(2R,3R,4S,5R)-5-[(R)-(3,4-difluorophenyl)-hydroxy-methyl]-3,4-dihydroxy-tetrahydrofuran-2-yl]pyrrolo[2,3-d]pyrimidin-4-yl]amino]propan-2-one (**12**)

A 20 mL vial containing (2R,3S,4R,5R)-2-[(R)-(3,4-difluorophenyl)-hydroxy-methyl]-5-[4-[(2-methyl-1,3-dioxolan-2-yl)methylamino]pyrrolo[2,3-d]pyrimidin-7-yl]tetrahydrofuran-3,4-diol (**12b**) (55 mg, 0.11 mmol) was charged with a RT solution of 2,2,2-trifluoroacetic acid;TFA (0.50 mL, 6.5 mmol) in Water (0.50 mL), then was purged with Ar for 3 min. The reaction was stirred at RT for 1 d. Reaction was completed by HPLC analysis. The reaction mixture was charged with a small amount of DMSO, loaded onto a 30 g C18 column, and purified by FCC (0 to 35% MeCN in H2O). Fractions containing pure product by HPLC were combined and co-evaporated twice under reduced pressure with 1 N HCl (aq) and MeCN. The sample was then co-evaporated thrice under reduced pressure with MeCN and water and heat (up to 50 °C) to yield 1-[[7-[(2R,3R,4S,5R)-5-[(R)-(3,4-difluorophenyl)-hydroxy-methyl]-3,4-dihydroxy-tetrahydrofuran-2-yl]pyrrolo[2,3-d]pyrimidin-4-yl]amino]propan-2-one hydrochloride (43.4 mg, 0.089 mmol, 78% yield) as a shiny, pale yellow powder. A portion of the HCl salt product was dissolved in MeOH, neutralized with Amberlite IRA-67 resin, and filtered. The filtrate was concentrated under reduced pressure and heat (50 °C) to yield 1-[[7-[(2R,3R,4S,5R)-5-[(R)-(3,4-difluorophenyl)-hydroxy-methyl]-3,4-dihydroxy-tetrahydrofuran-2-yl]pyrrolo[2,3-d]pyrimidin-4-yl]amino]propan-2-one (**12**) (12.5 mg, 0.0279 mmol, 24.231% yield) as a white powder. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.10 (s, 1H), 7.98 (s, 1H), 7.48 – 7.32 (m, 3H), 7.30 – 7.21 (m, 1H), 6.66 (dd, J= 13.2, 3.7 Hz, 2H), 5.94 (d, J= 7.8 Hz, 1H), 5.26 (d, J= 7.1 Hz, 1H), 5.06 (d, J= 4.0 Hz, 1H), 4.81 (t, J= 4.1 Hz, 1H), 4.61 (td, J= 7.4, 4.9 Hz, 1H), 4.28 (d, J= 5.8 Hz, 2H), 4.06 – 3.97 (m, 2H), 2.13 (s, 3H). LCMS (ESI) m/z calcd for $[M+H]^+$ $C_{20}H_{21}F_2N_4O_5$: 435.14; Found:435.2.

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